Generation and characterization of transgene-free human induced pluripotent stem cells and conversion to putative clinical-grade status.

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Public Summary:
Many diseases, injuries and even age-related tissue degeneration may eventually be treatable using human induced pluripotent stem cells (hiPSCs). Viral based reprogramming is still an often used and very robust methodology for expressing key genes associated with full nuclear reprogramming of an adult somatic cell back into an embryonic stem cell like pluripotent state. However, it is well known that most viral integrations occur into actively transcribed genes which could pose a safety concern due to potential oncogenic transgenic elements being actively and sporadically expressed after reprogramming. We reprogrammed adult somatic fibroblasts from a donor patient and derived multiple hiPSC lines that were propagated in vitro. We then used a highly sensitive PCR-based technique to analyze the vector-genome integration site and chose the clone that contained one genomic integration into an actively transcribed gene, PRPF39. We followed this by Cre-mediated excision to remove the lentivirus from the genome and fully characterized this new factor-free hiPSC line through characteristic pluripotency assays. Interestingly we observed that integration into PRPF39 caused aberrantly high gene expression that was ameliorated upon excision while still maintaining proper gene splicing. Important to clinical translation and future human clinical trials, these post-excised hiPSCs were differentiated into a variety of therapeutically relevant cell types including oligodendrocyte progenitor cells, hepatocytes, fibroblasts, and cardiomyocytes. All of this research, initially, was done in conditions that expose the cells to animal by-products. This is limiting in the sense that ideally no cellular therapeutics should be used that are maintained in conditions that present non-human associated antigens that would be potentially immunogenic. Therefore, we sought to follow a protocol similar to the one Geron Corporation implemented for their phase 1 clinical trial using embryonic stem cell-derived oligodendrocyte progenitor cells to treat spinal cord injury. To this end, the hiPSCs were cultured for extended periods of time in completely defined xeno-free conditions and then properly tested for sterility and other parameters as mandated by the FDA for Geron. Importantly, this paper introduces for the first time a methodology to analyze the post-converted xeno-free hiPSCs for a non-human sialic acid antigen exclusively expressed on non-human animal products. Since humans commonly have circulating antibodies to this antigen, it is imperative that hiPSCs that are acting as the starting material for clinically relevant therapeutic derivatives be void of any residual and immunogenic non-human sialic acids. We provide evidence for complete absence of this sialic acid upon extended culture in xeno-free conditions. For the first time these studies provide proof-of-principle for the generation of fully characterized transgene-free human iPSCs and highlights an attractive mechanism for converting research-grade cell lines into putatively clinical-grade biologics for personalized cellular therapeutics.

Scientific Abstract:
INTRODUCTION: The reprogramming of a patient's somatic cells back into induced pluripotent stem cells (iPSCs) holds significant promise for future autologous cellular therapeutics. The continued presence of potentially oncogenic transgenic elements following reprogramming, however, represents a safety concern that should be addressed prior to clinical applications. The polycistronic "stem cell cassette" (STEMCCA), an excisable lentiviral reprogramming vector, provides, in our hands, the most consistent reprogramming approach that addresses this safety concern. Nevertheless, most viral integrations occur in genes, and exactly how the integration, epigenetic reprogramming, and excision of the STEMCCA reprogramming cassette influences those genes and whether these cells still have clinical potential are not yet known. METHODS: In this study, we used both microarray and sensitive real-time polymerase chain reaction to investigate gene expression changes following both intron-based reprogramming and excision of the STEMCCA cassette

during the generation of human iPSCs from adult human dermal fibroblasts. Integration site analysis was conducted using non-restrictive linear amplification polymerase chain reaction. Transgene-free iPSCs were fully characterized via immunocytochemistry, karyotyping and teratoma formation, and current protocols were implemented for guided differentiation. We also utilized current good manufacturing practice guidelines and manufacturing facilities for conversion of our iPSCs into putative clinical grade conditions. RESULTS: We found that a STEMCCA derived iPSC line that contains a single integration, found to be located in an intronic location in an actively transcribed gene, PRPF39, displays significantly increased expression when compared to post-excised stem cells. STEMCCA excision via Cre recombinase returned basal expression levels of PRPF39. These cells were also shown to have proper splicing patterns and PRPF39 gene sequences. We also fully characterized the post-excision iPSCs, differentiated them into multiple clinically relevant cell types (including oligodendrocytes, hepatocytes, and cardiomyocytes), and converted them to putative clinical-grade conditions by using the same approach previously approved by the US Food and Drug Administration for the conversion of human embryonic stem cells from research-grade to clinical-grade status. CONCLUSIONS: For the first time these studies provide proof-of-principle for the generation of fully characterized transgene-free human iPSCs and, in light of the limited availability of current good manufacturing practice cellular manufacturing facilities, highlight an attractive potential mechanism for converting research-grade cell lines into putatively clinical-grade biologics for personalized cellular therapeutics.
